Human Papillomavirus Type 16 Infections and 2-Year Absolute Risk of Cervical Precancer in Women With Equivocal or Mild Cytologic Abnormalities

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Background: **The 2-year absolute risk for cervical precancer attributable to infection by human papillomavirus type 16 (HPV16), the most common and oncogenic HPV type, in the millions of women diagnosed annually with equivocal or mildly abnormal cytology has not been definitively evaluated.** ***Methods:*** **Baseline cervical specimens of 5060 women with equivocal (atypical squamous cells of undetermined significance [ASCUS]) or mildly abnormal (low-grade squamous intraepithelial lesion [LSIL]) cytology were tested for HPV DNA using Hybrid Capture 2 (HC2) and type-specific L1 consensus primer polymerase chain reaction. We calculated absolute risks with 95% confidence intervals (CIs) for cumulative diagnosis, during the 2-year study period, of cervical intraepithelial neoplasia grade 3 (CIN3) ($n = 535$) or cancer ($n = 7$) (collectively referred to as ≥CIN3) and compared risk by HPV16 status and by other oncogenic HPV types using logistic regression. All statistical tests were two-sided.** ***Results:*** **The baseline prevalences of HPV16 in women with ASCUS or LSIL cytology were 14.9% and 21.1%, respectively. Women with ASCUS or LSIL cytology who were HPV16 DNA positive at baseline had 2-year cumulative absolute risks for ≥CIN3 of 32.5% (95% CI = 28.4% to 36.8%) and 39.1% (95% CI = 33.8% to 44.7%), respectively. By comparison, women with ASCUS who were positive by HC2 for other oncogenic HPV types combined had an 8.4% (95% CI = 6.9% to 10.4%) risk for ≥CIN3, which was similar to the risk posed by having ASCUS (risk = 8.8%, 95% CI = 7.9% to 9.8%) without knowledge of the oncogenic HPV DNA status. Women with LSILs who were positive by HC2 for other oncogenic HPV types combined had a 9.9% (95% CI = 8.0% to 12.0%) 2-year risk for ≥CIN3, which was less than the risk posed by having LSILs (risk = 15.0%, 95% CI = 13.3% to 16.9%) without knowledge of the oncogenic HPV DNA status. Together, women with ASCUS or LSILs who were HPV16-positive had the highest 2-year risk for ≥CIN3 compared with women who were HPV-negative (odds ratio [OR] = 38, 95% CI = 22 to 68; $P < .001$), fivefold greater than the increased risk in women who were positive for other oncogenic HPV types (OR = 7.2, 95%CI = 4.2 to 13, $P < .001$).** ***Conclusions:*** **Distinguishing the high absolute risk for cervical precancer in HPV16-positive women from the lower risk posed by other oncogenic HPV types might have clinical implications. [J Natl Cancer Inst 2005;97:1066–71]**

Among women participating in cervical screening programs, approximately two-thirds of those with high-grade cervical neoplasia have antecedent equivocal or mild cytologic abnormalities *(1)*. Equivocal cytology, or ASCUS (atypical cells of undetermined significance), and LSILs (low-grade squamous intraepithelial lesions) are the most common non-normal cytologic findings, representing 4.0% and 2.1% *(2)*, respectively, of the

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60 million Pap tests performed annually in United States. Approximately 50% of ASCUS *(3,4)* and 80% of LSIL (mildly abnormal cytology) *(5)* interpretations are associated with infection by oncogenic types of human papillomavirus (HPV). It is now generally recognized that cervical infections by approximately 15 oncogenic HPV types cause virtually all cervical cancer and its immediate precursor (precancer), cervical intraepithelial neoplasia grade 3 (CIN3) *(6–8)*.

In the United States, oncogenic HPV DNA testing (or "triage") for patients with ASCUS has proven to be a useful alternative to referring patients for immediate colposcopy *(3)* to detect CIN3 and cancer (collectively referred to as ≥CIN3). Accordingly, HPV testing for a group of 13 oncogenic HPV types has now been approved in the United States for triage of patients with ASCUS cytology *(9–11)*. In contrast, oncogenic HPV DNA testing is not informative for triage of patients with LSILs *(5,12)* because nearly all LSIL patients are HPV positive. However, an alternative triage strategy for LSILs might be useful because it would be valuable to distinguish women with underlying ≥CIN3 from the majority of women with LSILs who have HPV infections that probably will clear on their own.

Large, international case–series and case–control studies have firmly established that approximately 50% of women with ≥CIN3 have HPV16 *(6,7,13)*. HPV16 is also the most common oncogenic HPV type among women in the general population, including women with ASCUS or LSILs. However, because of the lack of sufficiently large prospective studies, it has not been established how the absolute risk of ≥CIN3 differs by HPV16 status versus other oncogenic HPV types among women with equivocal or mildly abnormal cytology.

To examine this issue, we evaluated the 2-year cumulative absolute risks for ≥CIN3 attributable to baseline-detected oncogenic HPV infection. Specifically, we determined the risks attributable to baseline-detected HPV16 and other oncogenic HPV infection for women enrolled into ALTS (ASCUS LSIL Triage Study) *(12,14,15)* because of an ASCUS or LSIL Pap smear.

Subjects and Methods

Study Design and Population

ALTS was a randomized, multicenter clinical trial that compared three management strategies for 5060 women (median age = 25 years, interquartile range = 21–31 years, range = 18–81 years) with ASCUS (n = 3488) or LSILs (n = 1572): immediate colposcopy (referral to colposcopy regardless of enrollment test results) (IC arm); HPV triage (referral to colposcopy if the enrollment HPV result was positive or missing or if the enrollment cytology was high-grade squamous intraepithelial lesion [HSIL]) (HPV arm); and conservative management (referral to colposcopy if cytology at enrollment or during follow-up was HSIL) (CM arm). Women in all arms of the study were reevaluated by cytology every 6 months for 2 years of follow-up. An exit examination, with colposcopy scheduled for all women regardless of arm or prior procedures, was performed at 2 years. Women with histologically confirmed lesions that were CIN2 or more severe were treated by a loop electrosurgical excision procedure. Details on randomization, examination procedures, patient management, and laboratory and pathology methods have been described previously *(12,14,15)*. The National Cancer Institute and local institutional review boards approved the study, and all participants provided written informed consent.

HPV DNA Testing

Two HPV DNA tests were performed on clinical specimens collected at enrollment. Hybrid Capture 2 (HC2; Digene Corporation, Gaithersburg, MD) using probe set B, a pooled probe DNA test for one or more oncogenic HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68), was performed on specimens from 4819 [95.2% of the 5060 women in ALTS *(12,14,15)*]; a positive test does not identify the specific HPV type(s). L1 consensus primer PGMY09/11 polymerase chain reaction (PCR) amplification and reverse-line blot hybridization for detection of 27 individual HPV genotypes (HPV6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51–59, 66, 68, 73 [PAP238A], 82 subtype [W13b], 83 [PAP291], and 84 [PAP155]) *(16,17)* were performed on separate specimens from 4915 (97.1%) patients. For 2833 of these patients (58%), we tested for an additional 11 individual nononcogenic HPV genotypes (61, 62, 64, 67, 69–72, 81, 82 subtype [IS39], and 89 [CP6108]). Of the 5060 women enrolled into ALTS, 5052 (99.8%) women had at least one test result and 4682 (92.5%) had both test results. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 were considered the primary oncogenic HPV types *(6)* for this analysis.

Pathology and Treatment

Clinical management was based on the clinical center pathologists' cytologic and histologic diagnoses. In addition, all specimen slides were sent to the quality control pathology group (QC pathology), which was based at Johns Hopkins Hospital, for review and secondary diagnoses.

Statistical Analysis

We evaluated the 2-year absolute risk for histologically confirmed ≥CIN3 (n = 542, including seven cancers) diagnosed by QC pathology with binomial exact 95% confidence intervals (CIs) as stratified by the referral cytology. We also analyzed data at a less stringent disease diagnosis of ≥CIN2 as rendered by the individual ALTS clinical center pathologists (n = 932), because ≥CIN2 is the treatment threshold for ALTS following typical U.S. practice *(18)*. We evaluated cumulative risk at 2 years because all women had an exiting colposcopy for more complete disease ascertainment at 2 years; this endpoint allowed us to overcome any detection biases *(19)*, missed prevalent disease *(20)*, and/or differences between study arms.

Women were assigned a baseline HPV risk status according to the associations of HPV with cervical cancer *(7)*: PCR positive for HPV16 (HPV16+); any oncogenic HPV type positive, excluding HPV16 (oncogenic HPV positive by PCR or by HC2 but PCR negative for HPV16) (HPV16−/oncogenic HPV+); nononcogenic HPV positive (HC2 negative and PCR positive only for nononcogenic HPV types) (nononcogenic HPV+); and HPV negative (HPV−). The order of risk, from highest to lowest, was HPV16+>HPV−/oncogenic HPV+>nononcogenic HPV+>HPV−. As a point of reference, we calculated the absolute risk of ≥CIN2 and ≥CIN3 for any oncogenic HPV type, including HPV16 (oncogenic HPV+) as detected by PCR and/or by HC2. We separately evaluated the absolute risk for

≥CIN3 in women who were less than 30 years old and in women who were 30 years of age or older. The Pearson chi-square test was used to test for statistically significant differences ($P<.05$, two-sided) in absolute risk by age, by study arm, and by clinical center. Finally, we used logistic regression to calculate odds ratios (ORs) and 95% confidence intervals for ≥CIN3 associated with HPV risk status using combined HC2 and PCR data, referral Pap test interpretation, and age at enrollment, including interaction terms for HPV risk status and referral Pap test. Stata version 8.2 (Stata Corporation, College Station, TX) was used for all statistical analyses.

Results

Among women referred into the study, 48.0% (95% CI = 46.3% to 49.7%) and 71.3% (95% CI = 68.9% to 73.5%) of those with ASCUS or LSIL cytology were PCR positive for oncogenic HPV, respectively, and 53.1% (95% CI = 51.4% to 54.8%) and 84.1% (95% CI = 82.2% to 85.9%) were HC2 positive for oncogenic HPV, respectively. Differences in positivity for oncogenic HPV between PCR and HC2 may reflect differences in analytic sensitivity and the cross-reactivity of HC2 for certain nononcogenic HPV types *(21,22)*. HPV16 was the most common HPV type among women with ASCUS (14.9%, 95% CI = 13.7% to 16.1%) and among women with LSILs (21.1%, 95% CI =19.1% to 23.2%).

Overall, women with ASCUS or LSIL cytology had 2-year cumulative absolute risks for ≥CIN3 of 8.8% (95% CI = 7.9% to 9.8%) or 15.0% (95% CI = 13.3% to 16.9%), respectively (Table 1). Based on HC2 HPV testing of enrollment cervical specimens, oncogenic HPV+ women with ASCUS or LSILs had a 2-year absolute risk for ≥CIN3 of approximately 15% and 17%, respectively. HPV16−/oncogenic HPV+ women with ASCUS or LSIL had 2-year absolute risks for ≥CIN3 of approximately 8% and 11%, respectively. HPV− women with ASCUS or LSIL had 2-year absolute risks for ≥CIN3 of approximately 2% or 5%, respectively.

However, among HPV16+ women, the 2-year absolute risk for ≥CIN3 was 32.5% (95% CI = 28.4% to 36.8%) among women with ASCUS and 39.1% (95% CI = 33.8% to 44.7%) among women with LSILs. There were no statistically significant differences in the 2-year absolute risk for ≥CIN3 between single-type HPV16 infections and multiple-type HPV infections that included HPV16 for either women with ASCUS (36.8% for 136 women with single-type infections versus 31.0% for 365 women with multiple-type infections, $P = .2$) or women with LSILs (42.4% for 99 women with single-type infections versus 37.7% for 228 women with multiple-type infections, $P = .4$). The 2-year absolute risk estimates for ≥CIN3 by HPV status did not differ substantially when cancer diagnoses were excluded.

For women with other individual HPV types, the 2-year absolute risks for ≥CIN3 were lower; the risk in women with ASCUS ranged from 3.4% (HPV51) to 15.7% (HPV33), and the risk for women with LSILs ranged from 6.3% (HPV51) to 23.1% (HPV31). Among women positive for HPV18, the second most common type in cervical cancer, and negative for HPV16, the 2-year absolute risk of ≥CIN3 was 8.5% (95% CI = 4.3% to 14.7%) for those

Table 1. Absolute risks for clinical center pathology diagnosed CIN2 or more severe (≥CIN2) or quality control pathology diagnosed CIN3 or more severe (≥CIN3) for ASCUS- and LSIL Pap smear-referred women in different HPV risk groups defined by HC2 and PCR test results*

			≥CIN2		≥CIN3	
Test	HPV Risk Category	*N*	Risk, %	95% CI, %	Risk, %	95% CI, %
ASCUS						
All ASCUS		3488	15.3	14.1 to 16.5	8.8	7.9 to 9.8
HC2−	HPV−†	1559	3.0	2.2 to 3.9	1.4	0.9 to 2.1
HC2+	Oncogenic HPV+‡	1767	26.2	24.2 to 28.3	15.2	13.6 to 17.0
HPV16−§	HPV16−/oncogenic HPV+	1245	18.4	16.3 to 20.7	8.4	6.9 to 10.1
HPV16+§	HPV16+	443	48.5	43.8 to 53.3	34.3	29.9 to 38.9
PCR−	HPV−	1295	3.0	2.2 to 4.1	1.9	1.2 to 2.7
PCR+		2068	22.9	21.1 to 24.8	13.0	11.6 to 14.5
Nononcogenic	Non-oncogenic HPV+	454	9.0	6.6 to 12.1	3.3	1.9 to 5.4
Oncogenic	Oncogenic HPV+	1614	26.8	24.7 to 29.1	15.7	14.0 to 17.6
Oncogenic without HPV16	HPV16−/oncogenic HPV+	1113	18.4	16.2 to 20.8	8.2	6.6 to 9.9
HPV16+	HPV16+	501	45.5	41.1 to 50.0	32.5	28.4 to 36.8
LSIL						
All LSIL		1572	25.4	23.2 to 27.6	15.0	13.3 to 16.9
HC2−	HPV−†	237	8.4	5.2 to 12.7	4.6	2.3 to 8.2
HC2+	Oncogenic HPV+‡	1256	29.1	26.6 to 31.7	17.3	15.2 to 19.5
HPV16−§	HPV16−/oncogenic HPV+	931	21.6	19.0 to 24.4	9.9	8.0 to 12.0
HPV16+§	HPV16+	310	51.6	45.9 to 57.3	39.4	33.9 to 45.0
PCR−	HPV−	258	10.9	7.3 to 15.3	5.4	3.0 to 8.9
PCR+		1294	28.2	25.8 to 30.7	16.9	14.9 to 19.1
Nononcogenic	Non-oncogenic HPV+	188	11.2	7.0 to 16.6	3.2	1.2 to 6.8
Oncogenic	Oncogenic HPV+	1106	31.1	28.4 to 33.9	19.3	17.0 to 21.7
Oncogenic without HPV16	HPV16−/oncogenic HPV+	779	22.7	19.8 to 25.8	10.9	8.8 to 13.3
HPV16+	HPV16+	327	51.1	45.5 to 56.6	39.1	33.8 to 44.7

*We considered HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 to be the primary oncogenic HPV types *(6)* and other types to be nononcogenic. *CIN2/3 = cervical intraepithelial neoplasia grade 2/3; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HPV = human papillomavirus; HC2, Hybrid Capture 2; PCR = polymerase chain reaction; CI = confidence interval.

†Negative for oncogenic types; HC2 does not target nononcogenic HPV.

‡HC2 cross-reacts with some nononcogenic HPV types *(21)*.

§Based on polymerase chain reaction detection of HPV16.

with ASCUS and 14.5% (95% CI = 9.1% to 21.5%) for those with LSILs, similar to the pooled risk for HPV16−/oncogenic HPV+.

The 2-year absolute risks for ≥CIN3 by HPV status did not differ statistically significantly by study arm. For example, HPV16+ women with ASCUS assigned to the IC, HPV, and CM arms had absolute risks of 27.3%, 35.3%, and 35.5% for ≥CIN3, respectively (P = .2). HPV16+ women with LSILs assigned to the IC, HPV, and CM arms had absolute risks of 40.6%, 38.0%, and 38.3% for ≥CIN3, respectively (P = .9). The absolute risks for CIN3 by HPV status also did not differ statistically significantly by clinical center (data not shown).

The 2-year absolute risk estimate for clinical centers' diagnoses of ≥CIN2, the less stringent but clinically relevant endpoint for treatment *(18)*, was 45.5% (95% CI = 41.1% to 50.0%) among HPV16+ women with ASCUS and 51.1% (95% CI = 45.5% to 56.6%) among HPV16+ women with LSILs. We observed no appreciable differences in absolute risk estimates when using QC pathology's diagnoses (versus the clinical centers') of ≥CIN2 as an endpoint (data not shown).

Women with ASCUS who were younger than 30 years of age had a higher 2-year absolute risk for ≥CIN3 than did women 30 years and older who were PCR negative (risks = 2.9% versus 1.0%, P = .01), PCR positive for any HPV type (risks = 14.0% versus 9.6%, P = .01), or PCR positive for any nononcogenic HPV type (risks = 4.5% versus 0.7%, P = .03) (Table 2). Absolute risks for ≥CIN3 for LSIL-positive women were similar in both age groups.

In a multivariable model that included HPV risk status, referral Pap test interpretation, and age at enrollment (Table 3), HPV16 detection (HPV16+ versus HPV−, OR = 38, 95% CI = 22 to 68; P<.001) was the strongest risk factor for ≥CIN3 diagnosed during the 2-year trial. The risk associated with HPV16 was five times higher than that associated with other oncogenic types (OR = 7.4, 95% CI = 4.2 to 13; P<.001). Women with LSIL cytology or a nononcogenic HPV infection had an approximately threefold higher risk of ≥CIN3 diagnosis than did women with ASCUS cytology or those who were HPV negative, respectively. Overall, women 30 years of age and older were not at an elevated risk of ≥CIN3 compared with women younger than 30 years of age. Estimates of relative risk observed using a ≥CIN2 diagnosis by the clinical center pathologists as the endpoint were similar to those observed for ≥CIN3 diagnosed by QC pathology (data not shown).

Discussion

We demonstrated in this population of mostly young women with either equivocal or mildly abnormal cervical cytology that having a baseline, prevalent HPV16 infection (HPV16+) was associated with a very high absolute risk of ≥CIN3 over a 2-year period, a fivefold greater risk than the collective risk attributable to other prevalent oncogenic HPV type infections.

We observed an approximately 50% 2-year absolute risk of the clinically relevant ≥CIN2 for HPV16+ women with LSILs. This is consistent with a previous study of 455 women that found a 62% 3-year absolute risk of ≥CIN2 for HPV16+ women with a mildly abnormal Pap smear (mild to severe dyskaryosis) *(23)*.

The 2-year risk of ≥CIN3 among women with non-HPV16 oncogenic types (HPV16−/oncogenic HPV+) was similar to the risk

Table 2. Absolute risks for quality control pathology diagnosed CIN3 or more severe (≥CIN3) for ASCUS- and LSIL Pap test-referred women in different HPV risk groups stratified by age*

		<30 years of age			≥30 years of age			
Test	HPV Risk Category	N	Risk, %	95% CI, %	N	Risk, %	95% CI, %	P
ASCUS								
All ASCUS		2270	11.1	9.8 to 12.5	1,218	4.4	3.3 to 5.7	<.001
HC2−	HPV−†	731	1.9	1.1 to 3.2	828	1.0	0.4 to 1.9	.1
HC2+	Oncogenic HPV+‡	1423	15.9	14.0 to 17.9	344	12.5	9.2 to 16.5	.1
HPV16−§	HPV16−/oncogenic HPV+	980	8.8	7.1 to 10.7	265	7.2	4.4 to 11.0	.4
HPV16+§	HPV16+	383	33.9	29.2 to 38.9	60	36.7	24.6 to 50.1	.7
PCR−	HPV−	586	2.9	1.7 to 4.6	709	1.0	0.4 to 2.0	.01
PCR+		1600	14.0	12.3 to 15.8	468	9.6	7.1 to 12.7	.01
Nononcogenic	Non-oncogenic HPV+	310	4.5	2.5 to 7.5	144	0.7	0.0 to 3.8	.03
Oncogenic	Oncogenic HPV+	1290	16.3	14.3 to 18.4	324	13.6	10.0 to 17.8	.2
Oncogenic without HPV16	HPV16−/oncogenic HPV+	867	8.4	6.7 to 10.5	246	7.3	4.4 to 11.3	.6
HPV16+	HPV16+	423	32.4	27.9 to 37.1	78	33.3	23.1 to 44.9	.9
LSIL								
All LSIL		1304	15.5	13.6 to 17.6	268	12.7	8.9 to 17.3	.2
HC2−	HPV−†	170	4.7	2.1 to 9.1	67	4.5	0.9 to 12.5	.9
HC2+	Oncogenic HPV+‡	1071	17.4	15.1 to 19.8	185	16.8	11.7 to 22.9	.8
HPV16−§	HPV16−/oncogenic HPV+	786	10.1	8.0 to 12.4	145	9.0	4.9 to 14.8	.7
HPV16+§	HPV16+	275	38.2	32.4 to 44.2	35	48.6	31.4 to 66.0	.2
PCR−	HPV−	180	5.6	2.7 to 10.0	78	5.1	1.4 to 12.6	.9
PCR+		1111	17.1	14.9 to 19.4	183	15.8	10.9 to 22.0	.7
Nononcogenic	Non-oncogenic HPV+	159	3.1	1.0 to 7.2	29	3.4	0.1 to 17.8	.9
Oncogenic	Oncogenic HPV+	952	19.4	17.0 to 22.1	154	18.2	12.4 to 25.2	.7
Oncogenic without HPV16	HPV16−/oncogenic HPV+	662	11.3	9.0 to 14.0	117	8.5	4.2 to 15.2	.4
HPV16+	HPV16+	290	37.9	32.3 to 43.8	37	48.6	31.9 to 65.9	.2

*We considered HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68 to be the primary oncogenic HPV types *(6)* and other types to be nononcogenic. P values (two-sided) were calculated using the Pearson chi-square test. CIN3 = cervical intraepithelial neoplasia grade 3; HPV = human papilloma virus; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion; HC2 = hybrid capture 2; CI = confidence interval.

†Negative for oncogenic types; HC2 does not detect nononcogenic HPV.

‡HC2 cross-reacts with some non-oncogenic HPV types *(21)*.

§Based on polymerase chain reaction detection of HPV16.

Table 3. Odds ratios (OR) and 95% confidence intervals (95% CIs) for 2-year cumulative CIN3 ($n = 542$) and cancer ($n = 7$) (≥CIN3) diagnoses associated with HPV status, referral Pap test interpretation, and age at enrollment*

Characteristic	OR (95% CI)	*P*
HPV Risk Category		
HPV−	1.0 (referent)	
Nononcogenic HPV+	2.6 (1.2 to 5.6)	.02
HPV16−/oncogenic HPV+	7.4 (4.2 to 13)	<.001
HPV16+	38 (22 to 68)	<.001
Referral Pap test cytology		
ASCUS	1.0 (referent)	
LSIL	2.9 (1.1 to 7.7)	.03
Age at enrollment, y		
<30	1.0 (referent)	
≥30	0.86 (0.66 to 1.1)	.3

*CIN3 = cervical intraepithelial neoplasia grade 3; HPV = human papillomavirus; ASCUS = atypical squamous cells of undetermined significance; LSIL = low-grade squamous intraepithelial lesion. We considered HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 to be the primary oncogenic HPV types *(6)* and other types to be nononcogenic. HPV status was based on the combined results of Hybrid Capture 2 and polymerase chain reaction. *P* values (two-sided) were calculated using the Pearson chi-square test.

attributable to having an ASCUS Pap smear without consideration of HPV status. For no other single HPV type did the risk of ≥CIN3 approach that associated with HPV16. Of note, six of the seven women (median age = 36 years) diagnosed as having cancer were HPV16+. Although a large proportion of ≥CIN3 was identified at baseline, 238 cases (44%) of ≥CIN3 (91 HPV16+ cases) were detected during the 2-year follow-up and included two follow-up cancer cases that were both HPV16+ at baseline and were probably baseline cancers first detected at follow-up *(20)*. Thus, detection of HPV16 infection was the single most important risk factor for cancer and for missed ≥CIN3 in this population.

It should be noted that our precise estimates of absolute risk for combinations of cytology and HPV DNA testing are limited to the ALTS study population. Cytologic definitions of ASCUS of LSIL that include more severe cytology will probably result in greater risks associated with the different HPV risk strata. However, it seems unlikely that the relative importance of HPV risk strata will vary depending on the thresholds of cytologic interpretations. On the other hand, changes in the analytic sensitivity of HPV testing would probably affect both absolute and relative risk estimates by altering which women are identified as HPV positive. This connection between analytic sensitivity of an HPV test and the clinical performance is not linear. When analytic sensitivity is too low or too high, the risk estimates for cancer decrease *(24)*. These limitations regarding the extension of ALTS results to other populations do not change the general findings, but they do suggest the need for realistic effectiveness research if HPV typing is added to current clinical protocols.

Our results did not differ statistically by study arm or by clinical center, which we infer to mean that these findings are independent of study design, are robust, and can be generalized. The cytologic interpretation of the referral Pap smear, ASCUS versus LSIL, was only weakly associated with the risk of ≥CIN3. Thus, when calculating risk, it was far more informative to know a patient's HPV status than her cytologic ASCUS or LSIL interpretation.

It is noteworthy that women who were HPV negative by either HPV test had low, although nonzero, risk of ≥CIN3 over 2 years and that women with either ASCUS or LSILs who were negative by both tests had an approximately 1% risk of ≥CIN3 over 2 years (data not shown). We suggest that this residual risk for precancer is attributable to failure of cervical cell sampling, false-negative test results, or incident disease. These data highlight that no test or combination of tests will provide perfect negative reassurance for cervical precancer or cancer.

We observed that the presence of nononcogenic HPV types increased the absolute risk of ≥CIN2 compared with that in HPV-negative women, especially among women with ASCUS, but that the absolute risk for ≥CIN3 among nononcogenic HPV-infected women did not differ statistically from the risk among HPV-negative women.

We considered 13 types, HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68, as the primary oncogenic HPV types *(6)* and other types to be nononcogenic. A recent report has suggested that other types might also be oncogenic *(7)*. HPV53, an example of these potentially oncogenic types that we classified a priori as nononcogenic, was associated with only a 2.1% risk for ≥CIN3 in the absence of other oncogenic HPV types.

Therefore, our data indirectly reinforce the impression that CIN2 is heterogeneous (i.e., a mixture of productive infections, even by nononcogenic types, and cancer precursors) and that although CIN2, as the clinical threshold for ablative treatment, provides a margin of safety, CIN3 more correctly represents true precancer.

The currently Food and Drug Administration–approved HC2 test uses pooled probes for 13 oncogenic HPV types and does not distinguish individual types. Although it is useful for ASCUS triage, HC2 is not recommended for LSIL triage because the high proportion of positive results makes it uninformative. However, if the elevated risk of ≥CIN3 in HPV16+ ASCUS or HPV16+ LSIL (30%–40%) warrants more aggressive management (for example, immediate treatment in selected patients at risk of loss-to-follow-up), then separate HPV16 detection might be useful for the management of women with ASCUS or LSILs. A pooled-probe test and an HPV16 type-specific test could be performed concurrently or sequentially for ASCUS triage. A HPV16 type-specific test alone could be performed for LSIL "triage." In absolute numbers, women with HPV16+ ASCUS or LSILs represent a substantial number of patients, based on the frequency of these cytologic interpretations *(2)* and the prevalence of HPV16 within each cytologic category. From our data, approximately 500 000 women will have HPV16+ ASCUS or LSILs annually in the United States and may deserve more careful surveillance. Whether HPV16− (oncogenic HPV+) ASCUS or LSIL women can be safely managed less aggressively remains an important but unanswered clinical question.

In addition, among women who are diagnosed with less than CIN2–3 at colposcopy, knowledge of HPV16 status may have clinical utility in guiding postcolposcopy management by stratifying women according to their risk for subsequent high-grade cervical neoplasia. In ALTS, women with less than CIN2 at enrollment colposcopy but who were HPV16+ were far more likely to have a 2-year follow-up or exit diagnosis of ≥CIN3 (14.3%) compared with women who were oncogenic HPV+ but HPV16− (7.2%) ($P = .0005$). Although the difference is modest, stratification by HPV16 detection better distinguishes risk than the histologic distinction between negative and CIN1 results from a colposcopically directed biopsy *(20)*.

In the accompanying manuscript *(25)*, we demonstrate in a screening population the potential utility of adjunctive testing for HPV16 and possibly HPV18 individually along with pooled probe tests for oncogenic HPV. Based on these data, we suggest

that risk stratification using type-specific HPV16 detection (and perhaps HPV18 detection in screening) warrants further clinical, technical, and cost-effectiveness analyses.

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Notes

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